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Research Article

Are Rheumatoid Arthritis Patients More Vulnerable To Cardiovascular Disease?

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Abstract: Increased mortality rate because of Cardiovascular Disease [CVD] is well established in Rheumatoid Arthritis [RA] patients. We have estimated the role of serum malondialdehyde [MDA] and homocysteine levels of serum in RA patients and we correlated whether the biochemical parameters in RA patients are more vulnerable for cardiovascular disease or not. The method in this study Serum MDA and homocystein levels were measured in 28 diagnosed patients of RA in the age from 25 to 65 were compared with healthy controls. MDA was estimated by the method of Randox laboratory. Serum homocysteine was measured by using ELISA kits. The results were **S**erum MDA levels were higher RA patients than in controls [0.8928 + 1.2168 micromoles/L vs. 0.5688 + 0.4864 micromoles/L; [$p<0.05$]. There was significant [$p<0.05$] rise in serum homocsyteine level in patients than in controls [47.25 + 23.109 micromoles/Lvs16.648 + 9.627 micromoles/L]. There was statistical significant [$p<0.05$] lower hemoglobin than normal & raised ESR levels in the RA patients. Hence we conclude that RA patients had elevated levels of MDA and homocysteine than the control group and both these parameters are common mediator in the pathogenesis of accelerated atherosclerosis.
Keywords: Cardiovascular Disease, Rheumatoid Arthritis, Serum Homocysteine, Serum Malondialdehyde.

INTRODUCTION

Rheumatoid arthritis [RA] is an autoimmune disease that results in chronic, systemic inflammatory disorder that affects many tissues and organs, but principally involves the synovial joints. RA can produce inflammation in the lungs, pericardium, sclera and also nodular lesions, most common in subcutaneous tissue. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated.

The prevalence of RA is 1% of the world's population. Women are three times more likely to develop RA. Onset is most frequent between the ages of 40-50, but people of any age can be affected. Wide range of biochemical markers implicated directly or indirectly in pathogenesis of RA like homocysteine, adenosine deaminase [ADA], Malondialdehyde[MDA] [1].The evaluation of oxidative stress by MDA and homocysteine is receiving increased attention as they are an early marker of oxidative stress in humans.

MDA is a major reactive aldehyde used as an indicator of free radical induced tissue damage [2].There is persistent inflammation in the synovial membranes of joints, associated with migration of activated phagocytes and other leukocytes into synovial and periarticular tissue. During phagocytosis, monocytes, neutrophils and macrophages generate superoxide radicals, hydrogen peroxide and the highly reactive hydroxyl radicals. These cytotoxic reactive oxygen species [ROS] may cause oxidative damage in the cells, which are capable of destroying membrane lipids, proteins, deoxyribonucleic acid, hyaluronic acid and cartilage [3].

RA is associated with increased co-morbidity and mortality resulting from CVD. Homocysteine, which is an intermediate product of methionine catabolism, is considered as major risk factor for CVD [1].

Homocysteine degrades and inhibits the formation of the three main structural components of the artery, which are collagen, elastin and the proteoglycans. Homocysteine permanently degrades cysteine disulfide bridges and lysine amino acid residues in proteins, gradually affecting function and structure of proteins. Homocysteine is a 'corrosive' of long-living proteins, i.e., collagen or elastin, or lifelong proteins, i.e., fibrillin. RA patients are more vulnerable to CVD in comparison to osteoarthritis patients as well as general population [1].

With this background we estimated that the biochemical parameters like MDA, homocysteine, CRP, RF (Rheumatoid Factor), Erythrocyte Sedimentation Rate [ESR] & Hemoglobin [Hb] to substantiate the role

of MDA in rheumatoid arthritis, assess if these patients show changes in homocysteine levels and to correlate if RA patients are more vulnerable to CVD.

MATERIAL & METHODS

A hospital based case control study was carried out in patients of RA taken from the medicine OPD of Lata Mangeskar Hospital [LMH] and N.K.P. Salve Institute of Medical Sciences and Research Centre, Nagpur. The study was conducted after the approval of ethics committee and the research was sponsored by the department of research, N.K.P. Salve Institute of Medical Sciences and Research Centre. A Total of 28 diagnosed patients of RA in the age group ranging from 25 to 65 were included in the study. They were age matched with 25 normal healthy controls.

Approximately 5 ml of venous blood samples had been collected from patients in plain and EDTA bulbs from patients with RA and normal healthy individuals. Blood samples were centrifuged at 3000g for 10 minutes. MDA had been estimated by the method of Randox laboratory. This method was based on the fact that lipid peroxide condenses with 1 methyl-2 phenyl indole [MPI] under acidic conditions resulting in the formation of a red chromophore. To determine specifically lipid peroxide in plasma, proteins are precipitated to remove water-soluble MPI reactive substance. The level of lipid peroxide is expressed in term of malondialdehyde. Tetra methoxypropane, which is converted quantitatively to MDA, was used as standard. Serum homocysteine was measured by using Enzyme Linked Immuno-Sorbant Assay [ELISA] kits. Statistical analyses were evaluated by using t-test [Open epi-info].

Inclusion criteria: Diagnosed cases of rheumatoid arthritis and the patients were diagnosed by the criteria given by the American Rheumatic Association [ARA].

Exclusion criteria:

a. Pregnancy
b. Other types of arthritis
c. Chronic disorder of bone, liver and endocrine
d. Patients on long-term medication
e. Patients with pre-existing heart disease

Any patients not willing to co-operate after initial signing the informed consent were allowed to withdraw from the study.

OBSERVATION AND RESULT

The serum MDA levels in controls were [0.5688 + 0.4864micromoles/L] and in patients with RA were [0.8928 + 1.2168 micromoles/L] with a statistical significant value [$p<0.05$]. Table I: shows statistical significant [$p<0.05$] rise in serum homocsyteine level in patients with RA [47.25 + 23.109micromoles/L] than the controls [16.648 + 9.627 micromoles/L].

The mean hemoglobin [Hb] levels in the patients were also found to be statistical significantly [$p<0.05$] lower than that of the healthy controls. No significant change was found in total leukocyte count [TLC] in between patients and healthy controls. There was increased level of ESR in patients as compared to healthy controls with significance [$p<0.05$] as shown in table II.

The results of RF and C-reactive protein in 28 RA patients were shown in table III. There were 27 negative results out of 28 RA patients with 1 patient showing the test positive.

DISCUSSION

In the present study MDA levels were found to be significantly [$p<0.005$] elevated in the patients with RA compared to healthy controls [Table 1]. Our study is in accordance with the other studies [3-7] where higher MDA levels have been reported in patients with RA. It indicates increased oxidative stress, which is reflected by increased lipid per oxidation in peripheral blood of patients with RA. MDA, the product of lipid per oxidation reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. Increasing lipid per-oxidation causes cell damage by oxidative stress. Increased lipid per oxidation may occur as a result of imbalance between the scavenging mechanisms and free radicals generation process. So this increased lipid per oxidation impairs membrane functions by decreasing membrane fluidity and changing the activity of membrane-bound enzyme and receptors. The products of lipid per oxidation are harmful to most cells in the body and are associated with atherosclerosis.

Table-1: Showing the results of serum homo csyteine and serum MDA

Biochemical Parameters	GROUP-I Controls[n= 25] [Mean + S.D]	GROUP –II Patients[n= 28] [Mean + S.D]
Serum homocysteine [Micromoles/L]	16.648 + 9.627	47.25 + 23.109***
Serum MDA [Micromoles/L]	0.5688 + 0.4864	0.8928 + 1.2168***

***p-value for homo cysteine: 0.00004761, ***p-value for MDA: 0.00002264

However in a study by Kajanachumpol *et al.;* [8] reported no change in lipid per oxidation in RA patients but Ozkan Y *et al*.; [9] reported increased MDA levels that were not sufficient to conclude that there was an increase in oxidative stress in RA patients.

In the present study it reveals that serum homocysteine were found to be significantly [p<0.005] elevated in the patients with RA compared to healthy controls [Table 1]. Our study is in accordance with the studies [10-13] where higher homocysteine levels have been reported in patients with RA.

Homocsyteine an intermediate product of methionine catabolism, which is an independent marker for vascular endothelial damage, the patients are more prone to develop atherosclerosis that may precipitate acute myocardial infarction in near future. That might be a major risk factor for developing cardiovascular disease [CVD] and our study support the other studies such as Daniel H Soloman *et al.;* [14], A McEntegrart *et al.;* [15]. Thus hyper homocysteinemia has been known to exert its detrimental effects through induction of acute and chronic inflammation pathway such as endothelial dysfunction, leucocytes adhesion, oxidative stress and the reduction of nitric oxide bioavailability [16].

But in a study by Hoekstra M *et al.;* [17] found that the relation between homocysteine and cardiovascular morbidity and mortality has not been fully elicited.

The mean hemoglobin [Hb] levels in the patients were also found to be significantly [p<0.05] lower than that of the healthy controls [Table 2]. Levels of Hb are also reported to be low in earlier studies [18] and it may be because of that inflamed tissues in the joints release proteins that impact the body's ability to use iron and produce red blood cells, leading to a low red blood count. But contradiction to our study Akyol *et al.;* [19] found no differences between Hb values of rheumatic patients and healthy controls.

Table-2: Showing the results of Hemoglobin, ESR and TLC

Blood Investigations	GROUP-I Controls[n= 25] [Mean + S.D]	GROUP –II Patients[n= 28] [Mean + S.D]
Hemoglobin [Hb] [g/dl]	11.96+0.956	10.78+2.107***
Total leucocytes count [TLC] [absolute count/ Microlitre]	6976+1154	6207.14+1403.15
Erythrocyte sedimentation rate [ESR] [mm/hr]	9.64+3.52	18.87+ 8.27***

***p-value for Hemoglobin: 0.0002, ***p-value for ESR: 0.000069; p-value for TLC: 0.336

Table-3: Showing the results of Rheumatic Factor (RF) factor and C-reactive protein.

Blood Investigations	GROUP-I Controls[n= 25] [Mean + S.D]	GROUP –II Patients[n= 28] [Mean + S.D]
Rheumatic Factor (RF)	25 - negative	27 – negative 1 – positive
C Reactive Protein [CRP]	25 - negative	21- negative 7 – positive

Erythrocyte Sedimentation Rate [ESR] is a diagnostic test for accounting the severity of inflammation. The higher the sedimentation rate the greater is the inflammation. Our result increased level of ESR in patients as compared to healthy controls with significance. This indicates high degree of inflammation.

CONCLUSION

Cardiovascular disease often goes unrecognized in patients with RA; therefore, it is prudent to carefully assess all patients with RA shortly after the diagnosis of RA is made. So on keeping a careful observation and analysis of the parameters of oxidative stress and homocysteine can help in assessment of cardiovascular status and improvement of health status in RA patients. Hence we conclude that immune activation involved in both oxidative stress and inflammation forms a vicious cycle and is common mediator in the pathogenesis of accelerated atherosclerosis and antioxidant therapy and monitoring of the patients for lipid profile and cardiovascular risk assessment can be undertaken in patients of early diagnosed of RA to prevent cardiovascular episodes in later life.

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